

IN THE SPECIFICATION

**[0036]** Turning to Figure ~~4~~2, a preferred process for the formation of mono- and bi-layer amphiphilic vesicles 110 and 112 is shown. First, a sheath flow 125 of two types of solutions is formed at the junction 115 of three (3) inlet microchannels 122, 123 and 124 of a microfluidic device 120. As depicted, the sheath flow can be an immiscible fluid sheath flow such as an oil(O)-water(W)-oil(O) flow or a water-oil-water flow. The sheath flow can comprise a three layered sandwiched flow. The relative flow rates of the oil and water through the microchannels 122, 123 and 124 are preferably controlled to dictate the viscous forces at the immiscible fluid or oil-water interfaces ~~427~~ 130 and 129, which determine droplet formation rate and size. Second, by dissolving amphiphiles 150 in the oil solutions 155 a monolayer membrane 109 is formed around or encapsulates the droplets 108 to form monolayer vesicles 110. The monolayer vesicle 110 can then be delivered across another oil-water interface 130 to form a bilayer vesicle 112 upon entering the aqueous solution 114.

**[0037]** As depicted, the central inlet microchannel 123 is an input microchannel for an aqueous solution 145 preferably comprising reagents 140. The reagents 140 can be of any form known in the art. In one embodiment, the reagents 140 include drugs or medicants which have been dissolved in an aqueous solution 145. Preferably the aqueous solution 145 comprises purified DI water(W).

**[0060]** The angle of entry  $\theta_A$  and  $\theta_B$  for the two inlet oil flow microchannels (122 and 124, 222 and 224, 322 and 324) relative to the inlet aqueous solution microchannel (123, 223 and 323) is generally in the range from about 0 to about 90 degrees (Figures 2, ~~3A-C~~, and 4A-B). Preferably, the angle of entry  $\theta_A$  and  $\theta_B$  for the two inlet oil flow microchannels (122 and 124, 222 and 224, 322 and 324) relative to the inlet aqueous solution microchannel (123, 223 and 323) is between about 10 and 60 degrees and most preferably is in the range of approximately 25 to 40 degrees. The angle of entry  $\theta_A$  and  $\theta_B$  for both oil flow microchannels (122 and 124, 222 and 224, 322 and 324) relative to the aqueous solution microchannel (123, 223 and 323) can be equal.

Alternatively, the angle of entry  $\theta_A$  and  $\theta_B$  for one of the two oil flow microchannels (122 and 124, 222 and 224, 322 and 324) can be greater than the angle of entry  $\theta_A$  and  $\theta_B$  for the other oil flow microchannel (122 and 124, 222 and 224, 322 and 324).

**[0063]** The “cross-junction device” (FIGS. 6A-6C) generates droplets at 1-2 drops/sec whereas the bowtie device (FIGS. 7A-7C) was much faster at 90 drops/sec. The cross-junction droplet generation rate can be increased in the cross-junction generator with higher flow rates.

**[0066]** As depicted in Figure 13, after formation, the monolayer vesicle 110 is delivered across an oil-water interface 130 to form a bilayer vesicle 112 upon entering the aqueous solution 114. The oil-water interface 130 is formed by water or an aqueous solution flowing through two water inlet channels 132 and 134 and an outlet channel 138. The amphiphilic molecules 135 of the oil-water interface 130 may be the same

type of amphiphilic molecules 150 as in the oil microchannel solvent 155. It is also possible to generate asymmetry in the bi-layers such that the inner and outer amphiphilic molecules are different. This is significant since biological membranes are, in fact, a fluid composition of lipids and other amphiphilic molecules. It will be possible to dynamically load the bi-layer amphiphilic vesicles 112 with different molecules, both in the membrane and encapsulated within it. Asymmetric vesicles are created through post encapsulating monolayer coated vesicles 110 with a second or more layers of encapsulating agents including alginate, lipids, polymer, surfactants and the like. For example, as depicted in Figures 13 and 14, an oleic acid coated monolayer vesicle 110 may be encapsulated by alginate gels 113 to form a hard shell around the permeable oleic acid monolayer 109. These vesicles will allow multiple drugs to be encapsulated inside a single unit providing a platform for a multiple drug delivery system.

**[0072]** Droplets of different sizes may also be sorted as shown in Figures 20A and 20B ~~21A and 21B~~. In this embodiment, droplets may be sorted by having an input channel 262 meet two daughter channels 264 and 266 at a T-junction. The streams 268 which direct a droplet to the lower daughter channel 264 or upper daughter channel 266 may be modified based on the relative flow rates of the two daughter channels 264 and 266. In Figure 20A, the complete suspension of the droplet 267 in the lower stream moves the droplet 267 to the bottom channel 264. If the flow rate of lower channel 264 is increased, the number of streams 268 moving toward the lower channel increases

(Figure 20B). This increases the droplet size that can be removed by the velocity gradient between the upper daughter channel 266 and the lower daughter channel 264.

**[0073]** Satellite droplets 281 can be separated from larger droplets 284 as shown in Figures 21A-22C. Satellite droplets 281 of less than 1  $\mu\text{m}$  in diameter can be generated, separated and then collected in the microchannel without using surfactants. Large and small satellite droplets are first generated in the microchannel (Figure 21A). The arrow indicates the direction of flow and the dash circles indicate the presence of a satellite droplet 281. Satellite droplets 281 are carried by the flow into the upper channel 288, while large droplets 284 are pulled by shear and pressure forces into the lower channel 285 (Figure 21B). Only satellite droplets 281 are observed in the collecting zone 289 for the upper channels 288 (Figure 21C). The flow rates used for this example were 1.7  $\mu\text{L}/\text{min}$  for water and 3.65  $\mu\text{L}/\text{min}$  for oil.